Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 154–158, January 1991
Genetics

Evidence for the evolutionary origin of human chromosome 21 from comparative gene mapping in the cow and mouse

(hybrid somatic cell analysis/*Bos taurus*/*Mus musculus*)

DEBORAH S. THREADGILL*, JAN P. KRAUS†, STEPHEN A. KRAWETZ‡, AND JAMES E. WOMACK*§

*Department of Veterinary Pathology, Texas A&M University, College Station, TX 77843; †Department of Pediatrics and Cellular/Structural Biology, University of Colorado Health Sciences Center, 4200 East Ninth Avenue, Denver, CO 80262; and ‡Department of Molecular Biology and Genetics, School of Medicine, Gordon H. Scott Hall, Wayne State University, 540 East Canfield, Detroit, MI 48202

Communicated by Neal L. First, October 1, 1990 (received for review July 27, 1990)

ABSTRACT **To determine the extent of conservation between bovine syntenic group U10, human chromosome 21 (HSA 21), and mouse chromosome 16 (MMU 16), 11 genes were physically mapped by segregation analysis in a bovine–hamster hybrid somatic cell panel. The genes chosen for study span MMU 16 and represent virtually the entire q arm of HSA 21. Because the somatostatin gene (*SST*), an HSA 3/MMU 16 locus, was previously shown to be in U10, the transferrin gene (*TF*), an HSA 3/MMU 9 marker, was also mapped to determine whether U10 contains any HSA 3 genes not represented on MMU 16. With the exception of the protamine gene *PRM1* (HSA 16/MMU 16), all of the genes studied were syntenic on bovine U10. Thus, all homologous loci from HSA 21 that have been studied in the cow are on a single chromosome. The bovine homolog of HSA 21 also carries several HSA 3 genes, two of which have homologous loci on MMU 16. The syntenic association of genes from the q arm of HSA 3 with HSA 21 genes in two mammalian species, the mouse and the cow, indicates that HSA 21 may have evolved from a larger ancestral mammalian chromosome that contained genes now residing on HSA 3. Additionally, the syntenic association of *TF* with *SST* in the cow permits the prediction that the rhodopsin gene (*RHO*) is proximal to *TF* on HSA 3q.**

Human chromosome 21 (HSA 21) has been the focus of intensive genetic mapping because of its association with Down syndrome. Genes mapped on HSA 21 include phosphoribosylglycinamide synthetase (*PRGS*); superoxide dismutase, soluble (*SOD1*); phosphoribosylaminoimidazole synthetase (*PAIS*); amyloid β-protein precursor (*APP*); phosphofructokinase, liver type (*PFKL*), α- and β-interferon receptor genes (*IFNRA/IFNRB*); avian erythroblastosis virus E26 oncogene homolog 2 (*ETS2*); α-crystallin polypeptide 1 (*CRYA1*); cystathionine β-synthase (*CBS*); S100 protein β polypeptide (*S100B*); lymphocyte function-associated antigen 1 (*CD18*); and type VI collagen, α1 and α2 (*COL6A1* and *COL6A2*) (compiled in ref. 1). Genes homologous to several of these HSA 21 genes, including *Sod-1*, *App*, *Ets-2*, *Pais*, *Prgs*, and *Ifnra/Ifnrb*, are located on the distal portion of mouse chromosome 16 (MMU 16). HSA 21 and MMU 16 are not completely conserved, however, in that Crya-1 and *Cbs* map to MMU 17, and *S100b*, *Cd18*, *Col6a1*, and *Col6a2* are on MMU 10 (compiled in ref. 2). Thus, genes on HSA 21 have homologs on three mouse chromosomes, MMU 10, 16, and 17. Additionally, MMU 16 contains homologs of genes present on three other human chromosomes, the protamine genes (*PRM1* and *PRM2*) on HSA 16, the λ light-chain immunoglobulin genes (*IGL*) on HSA 22, and somatostatin (*SST*) and growth-associated protein (*GAP43*) genes on HSA 3 (2). Unfortunately, extensive mapping of HSA 21 homologs has not been reported for any species other than the mouse. In the absence of greater comparative mapping data, the ancestral relationship of HSA 21 and MMU 16 cannot be resolved. Mapping of the loci from HSA 21 and MMU 16 in a third mammalian taxon such as the bovine, however, could provide important information about the evolution of HSA 21 and MMU 16 from an ancestral mammalian chromosome. The three species are thought to have been separated from each other for 80 million years, and genes syntenic in two of the three species are assumed to represent the ancestral arrangement.

Previous studies using bovine–hamster hybrid somatic cells showed the HSA 21 loci *SOD1*, *PRGS*, *PAIS*, *IFNRB*, and *CRYA1* to be syntenic in cattle (3–5). This bovine syntenic group, unassigned syntenic group 10 (U10), was later shown to contain *SST* (6). The synteny of *SST*, a HSA 3 marker, with the HSA 21 homologs is conserved on MMU 16. The homolog of *CRYA1*, however, is not found on MMU 16 but maps to MMU 17. Thus it appears that the evolution of HSA 21 and MMU 16 is more complex than a single rearrangement of one of the two chromosomes. To further examine the relationship between HSA 21, MMU 16, and bovine U10, the following genes were assigned to their respective bovine syntenic groups: *APP* and *ETS2* (HSA 21, MMU 16); *S100B*, *COL6A1*, *COL6A2*, and *CD18* (HSA 21, MMU 10); *CBS* (HSA 21, MMU 17); *PFKL* (HSA 21, MMU ?); *PRM1* (HSA 16, MMU 16); and *GAP43* (HSA 3, MMU 16) (2, 7–9). Additionally, the transferrin gene (*TF*), which is proximal to *SST* on HSA 3 (10), was mapped to determine whether U10 included HSA 3q genes not present on MMU 16. *TF* has been localized to MMU 9 in a conserved syntenic group with aminoacylase (*ACY1*) (11).

MATERIALS AND METHODS

Genomic DNA Preparation. Development and characterization of the bovine–hamster hybrid somatic cell panel have been described (12, 13). Genomic DNA was prepared from the hybrid somatic cell lines, the Chinese hamster cell line E-36, and bovine leukocytes by standard procedures (14). DNAs were digested with restriction endonucleases, electrophoresed in 1% agarose gels, and blotted to nylon membranes (Zetabind; AMF Cuno) by established techniques (14).

Probe Preparation. The 11 sequences utilized as hybridization probes have been described (Table 1). The plasmid inserts were released by digestion with the appropriate restriction enzymes and purified from agarose gels by electroelution (14). All insert DNAs were labeled with [α-^{32}P]dCTP

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: HSA *n*, human chromosome *n*; MMU *n*, mouse chromosome *n*; RFLP, restriction fragment length polymorphism.

§To whom reprint requests should be addressed.

Table 1. DNA sequences used as hybridization probes

Gene	Clone name	Clone type	Insert size, kb	Ref.
APP	pDS10.1	Mouse cDNA	1.2	15
ETS2	p1.27	Mouse genomic	1.27	16
GAP43	pGA3A	Human cDNA	1.0	17
PFKL	pG-PFKL 3.3	Human genomic	3.3	18
COL6A1	pML 18	Human cDNA	2.1	19
COL6A2	pML 1	Human cDNA	2.5	19
S100B	pSB	Rat cDNA	0.264	20
CD18	p3.1.1	Human cDNA	1.8	21
CBS	p610	Rat cDNA	1.7	22
PRM1	pBPK59	Bovine cDNA	0.317	23
TF	pTf66G2	Human cDNA	1.0	24

kb, Kilobases.

to specific activities $>10^9$ dpm/μg by the random primer method (25).

Southern Hybridization. Prehybridizations were at 42°C for 2 hr in 5× SSC/10× Denhardt's solution/0.05 M sodium phosphate, pH 7.0/5% (wt/vol) dextran sulfate/50% (vol/vol) formamide/0.1% (wt/vol) SDS containing sheared salmon sperm DNA (500 μg/ml). (SSC, standard saline citrate, is 0.15 M NaCl/0.015 M sodium citrate, pH 7; Denhardt's solution is 0.02% Ficoll/0.02% polyvinylpyrrolidone/0.02% bovine serum albumin.) Hybridizations were at 42°C for 18 hr in 5× SSC/1× Denhardt's solution/0.02 M sodium phosphate, pH 7.0/10% dextran sulfate/50% formamide/0.5% SDS containing sheared salmon sperm DNA (100 μg/ml) and labeled probe (5–10 × 10^6 cpm). For *CD18*, *ETS2*, *PFKL*, and *TF*, 40% formamide was substituted in the prehybridization and hybridization mixtures. Final washes were to a stringency of 1× SSC at 60°C for *CD18*, *COL6A1*, *COL6A2*, *ETS2*, *PFKL*, and *TF*, and 0.5× SSC at 65°C for *APP*, *CBS*, *GAP43*, *S100B*, and *PRM1*. Filters were placed against Kodak XAR-5 film with one DuPont Cronex Lightning Plus intensifying screen at −70°C for 1–7 days.

RESULTS

Homologs of 11 loci from either HSA 3, HSA 21, or MMU 16 were assigned to their respective bovine syntenic groups by using bovine–hamster hybrid somatic cells. Representative lanes from autoradiographs for all the genes mapped in this study are presented in Figs. 1 and 2*C*. Bovine-specific restriction fragments are easily discriminated from bands representing hamster homologs. *PRM*, the only bovine probe, did not cross-hybridize to hamster DNA under the conditions used (Fig. 1*I*), but does cross-hybridize to other more closely related species (8). Probes for two type VI collagen genes were hybridized simultaneously to DNAs from bovine–hamster hybrid somatic cells (Fig. 2*C*), as the two probes did not cross-hybridize under the conditions used (Fig. 2 *A* and *B*). However, an unusual segregation pattern was seen for the *COL6A1* bovine-specific bands (Fig. 2*C*). A restriction fragment length polymorphism (RFLP) was suspected, as more than one bovine leukocyte donor was used in the production of the bovine–hamster hybrid somatic cell panel. The *COL6A1* probe did detect a simple two-allele RFLP in *Bam*HI-digested bovine genomic DNAs (Fig. 2*D*).

A pairwise concordancy analysis of the 11 genes indicated that only *PRM1* (HSA 16, MMU 16) was asyntenic (Table 2). The segregation of *APP*, chosen to represent the 10 genes segregating concordantly, was compared to a representative marker from each of the 26 identified bovine syntenic groups (Table 3). Concordancy analysis revealed that *APP* segregated concordantly only with *SOD1* of bovine syntenic group U10. Thus, the other cosegregating genes, *ETS2*, *PFKL*, *S100B*, *COL6A1*, *COL6A2*, *CD18*, *CBS*, *GAP43*, and *TF*, are also in U10. This group contains *CRYA1*, *IFNRB*, *PAIS*, *PRGS*, and *SST* (3–5). *PRM1* was also compared to markers from the identified bovine autosomal syntenic groups and was 94% concordant with the HSA 7 marker malate dehydrogenase, mitochondrial (*MDH2*), of syntenic group U8 (12,

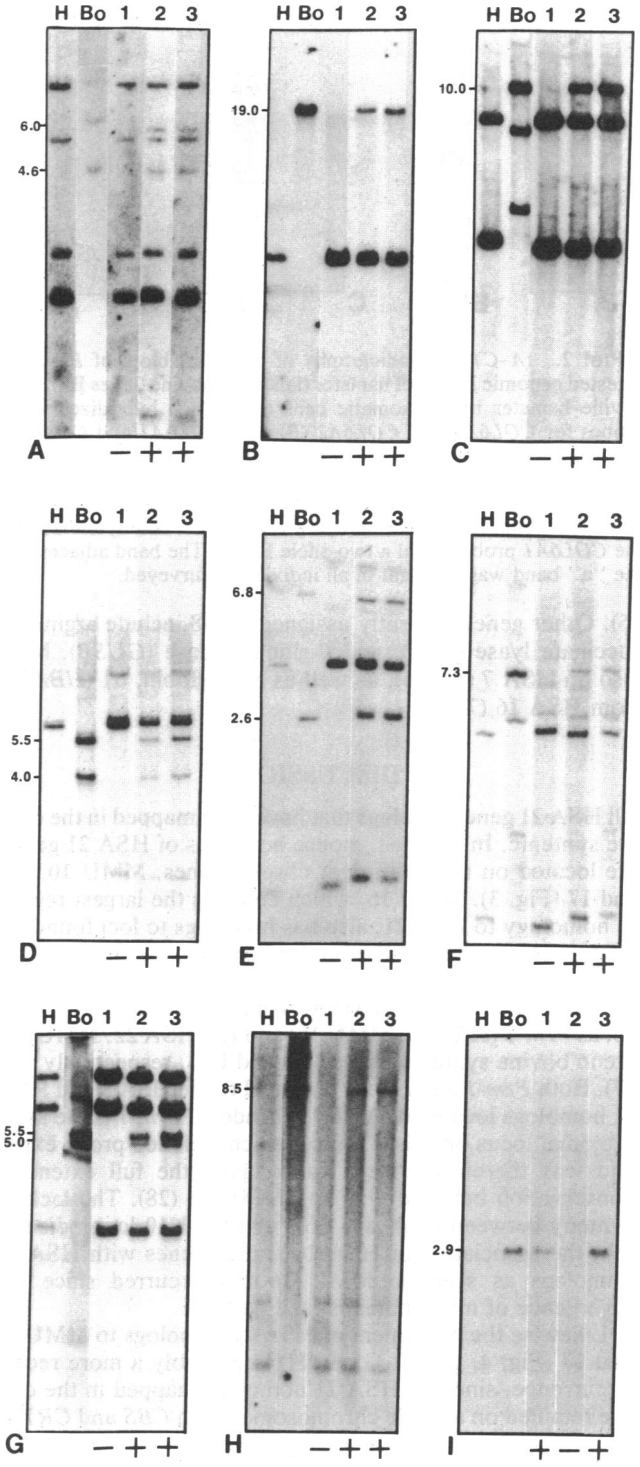

FIG. 1. Representative autoradiographs of Southern blots of genomic DNAs of hamster (lanes H), bovine (lanes Bo), and bovine–hamster hybrid somatic cells (lanes 1–3) hybridized with probes for *APP* (*A*), *ETS2* (*B*), *S100B* (*C*), *CD18* (*D*), *GAP43* (*E*), *TF* (*F*), *CBS* (*G*), *PFKL* (*H*), and *PRM1* (*I*). DNAs were digested with *Pst* I (*A*, *E*, and *F*), *Eco*RI, (*B*, *G*, and *I*), *Bgl* II (*C* and *H*), or *Bam*HI (*D*). Clones containing the homolog of the probe DNAs are indicated as + below the hybrid cell lanes, and the sizes of the bovine specific restriction fragments (kb) are indicated at left.

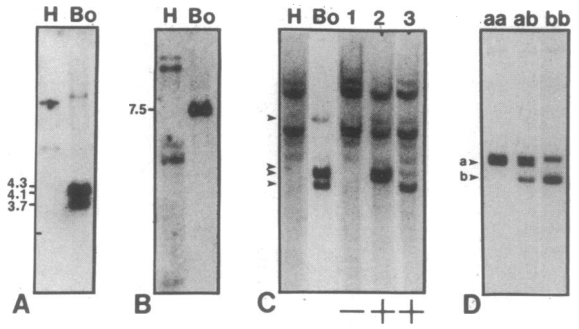

FIG. 2. (*A–C*) Autoradiographs of Southern blots of *Bam*HI-digested genomic DNAs of hamster (lanes H), bovine (lanes Bo), and bovine–hamster hybrid somatic cells (lanes 1–3) hybridized with probes for *COL6A1* (*A*), *COL6A2* (*B*), and *COL6A1* and *COL6A2* (*C*). Clones carrying the *COL6A1* and *COL6A2* bovine homologs are indicated as + below the hybrid-cell lanes, and the size(s) of the bovine specific restriction fragments (kb) are indicated at left. (*D*) Bovine genomic DNAs cut with *Bam*HI, blotted, and hybridized to the *COL6A1* probe reveal a two-allele RFLP. The band adjacent to the "a" band was constant in all individuals surveyed.

26). Other genes currently assigned to U8 include argininosuccinate lyase (*ASL*), and β-glucuronidase (*GUSB*), both also on HSA 7 (13, 26), as well as hemoglobin, α1 (*HBA1*), from HSA 16 (7, 13).

DISCUSSION

All HSA 21 gene homologs that have been mapped in the cow are syntenic. In contrast, mouse homologs of HSA 21 genes are located on three different chromosomes, MMU 10, 16, and 17 (Fig. 3). MMU 16, which contains the largest region of homology to HSA 21, also has homologs to loci found on HSA 3, 16, and 22 (2). The bovine homolog of HSA 21, U10, carries the HSA 3 genes found on MMU 16 (Fig. 4, group E), but it does not contain the HSA 16 or 22 genes found on MMU 16, as *Prm-1* (HSA 16, MMU 16) and *Igl* (HSA 22, MMU 16) are in bovine syntenic groups U8 and U23, respectively (13, 27). Both *Prm-1* and *Igl* are proximal to the HSA 3 and HSA 21 homologs located on MMU 16. Indeed, *Prm-1* is the most proximal locus on MMU 16 for which a cloned probe exists and was therefore chosen to compare the full extent of conservation between U10 and MMU 16 (28). The lack of synteny between *PRM* and *IGL* and the U10 loci indicates that the association of HSA 16 and 22 genes with HSA 21 homologs as seen on MMU 16 has occurred since the divergence of mice, humans, and cattle.

Likewise the movement of HSA 21 homologs to MMU 10 and 17 (Fig. 4, groups G and H) is probably a more recent occurrence, since all HSA 21 homologs mapped in the cow are retained on a single chromosome. Both *CBS* and *CRYA1*

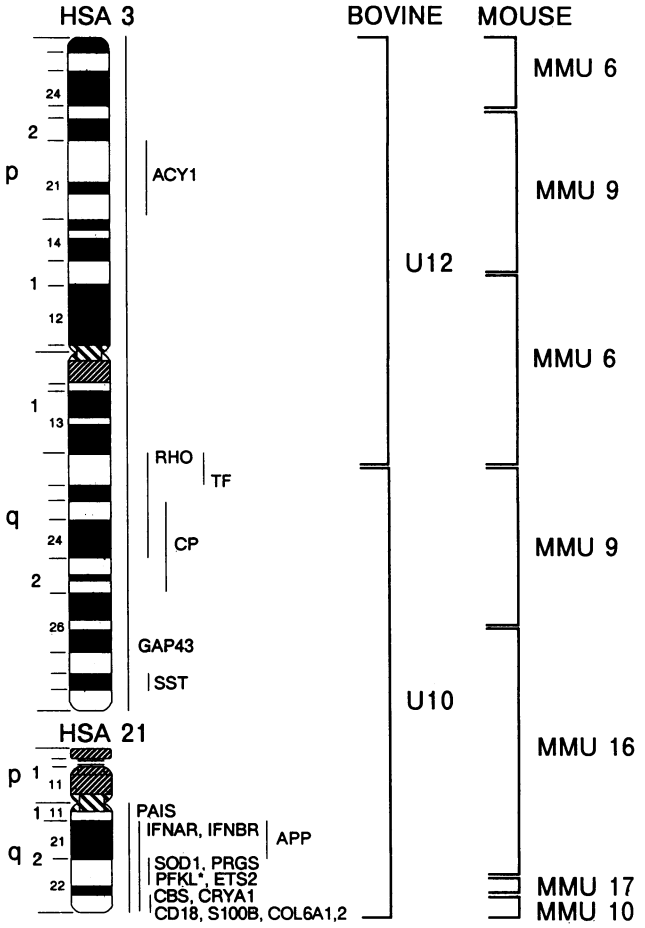

FIG. 3. Comparison of the chromosomal location of HSA 3 and HSA 21 loci in the cow and the mouse. Asterisk denotes a locus not mapped in the mouse.

are represented on MMU 17 and map to HSA 21q22.3. Other genes that map to this same Giemsa-negative band on HSA 21, such as *S100B*, *CD18*, *COL6A1*, and *COL6A2*, map to MMU 10 (1, 2). These loci are distal to the HSA 21 loci that map to MMU 16, indicating that the distal portion of the ancestral progenitor of HSA 21 was broken at least twice in the mouse lineage (Fig. 4).

In addition to the direct mapping of three HSA 3q loci (*SST*, *GAP43*, and *TF*) to bovine syntenic group U10, the previously reported linkage of *TF* and ceruloplasmin (*CP*) in the cow (29) allows the placement of *CP* in U10 as well. The syntenic association of four HSA 3q loci with HSA 21 loci as seen in the cow is similar to the situation seen on MMU 16, where two of the four loci (*GAP43* and *SST*) are syntenic with

Table 2. Pairwise concordancy analysis

	Clones tested	Clones positive	% retention	% concordant									
				PRM1	*TF*	*S100B*	*PFKL*	*GAP43*	*ETS2*	*COL6A2*	*COL6A1*	*CD18*	*CBS*
APP	36	16	44	57	100	100	100	100	100	97	100	100	100
CBS	34	16	47	52	100	100	100	100	100	100	100	100	
CD18	34	13	38	58	100	100	100	100	100	96	100		
COL6A1	37	15	41	59	100	100	100	100	100	97			
COL6A2	37	14	38	62	96	97	100	97	97				
ETS2	36	15	42	58	100	100	100	100					
GAP43	34	15	44	55	100	100	100						
PFKL	29	11	38	60	100	100							
S100B	35	15	43	59	100								
TF	28	10	36	55									
PRM1	35	10	29										

Table 3. Comparison of segregation of *APP* and *PRM1* with 26 identified bovine autosomal syntenic groups

	APP					*PRM1*				
	Concordant		Discordant			Concordant		Discordant		
Syntenic group	+/+	−/−	+/−	−/+	% concordant	+/+	−/−	+/−	−/+	% concordant
U1 (*PGD*)	10	16	2	2	87	5	16	2	6	72
U2 (*SOD2*)	10	12	4	8	65	6	13	3	11	58
U3 (*GAPD*)	11	12	1	5	79	4	10	3	11	50
U4 (*MPI*)	4	19	10	1	68	2	20	7	3	69
U5 (*PKM2*)	4	11	10	8	45	6	18	3	6	73
U6 (*PGM1*)	0	18	14	2	53	1	23	8	1	73
U7 (*LDHA*)	2	19	12	0	64	0	23	9	2	68
U8 (*MDH2*)	3	15	11	4	55	7	24	2	0	24
U9 (*GPI*)	0	17	14	3	50	0	21	9	3	64
U10 (*SOD1*)	14	20	0	0	100	2	17	5	9	58
U11 (*ITPA*)	8	12	4	4	71	4	13	3	7	63
U12 (*ACY1*)	3	12	11	8	44	5	18	4	6	70
U13 (*HOX1*)	1	17	14	1	55	1	20	9	1	68
U14 (*GSR*)	4	16	10	4	59	2	18	7	6	61
U15 (*PGM2*)	0	20	14	0	59	0	25	9	0	74
U16 (*ABL*)	9	12	7	7	60	4	11	5	19	38
U17 (*IDH1*)	4	18	10	2	65	3	21	6	3	73
U18 (*ACO1*)	3	20	10	0	70	1	22	8	2	70
U19 (*CAT*)	10	18	4	2	82	4	16	5	8	61
U20 (*GLO1*)	3	14	11	6	50	2	17	7	7	58
U21 (*GH*)	0	20	16	0	56	0	25	10	0	71
U22 (*AMH*)	0	19	16	0	54	0	25	9	0	74
U23 (*ALDH2*)	6	4	7	13	33	4	7	5	14	37
U24 (*MOS*)	11	18	4	2	83	4	16	6	8	59
U25 (*CLTLA1*)	5	5	8	11	34	7	9	2	10	57
U26 (*GOT1*)	0	18	14	0	56	0	22	10	0	69

certain HSA 21 loci (Fig. 3). The extant association of certain HSA 3q and HSA 21 genes in two diverse mammalian species supports the hypothesis of an ancestral syntenic relationship for these genes. Thus based on comparative mapping data, we propose that HSA 21 evolved from an ancestral mammalian chromosome that carried genes now residing on HSA 3 (Fig. 4). Although the gene order for loci residing on U10 and U12 (the bovine homolog of HSA 3) is unknown, this is the most parsimonious model consistent with the bovine synteny data and the human and mouse gene maps. More extensive synteny mapping of HSA 3 loci in the cow provides additional support for this hypothesis (D.S.T. and J.E.W., unpublished work).

In support of an ancestral association of HSA 3q markers with HSA 21 genes is the finding of a glutathione peroxidase 1-like (*GPXP1*) sequence on HSA 21 (30), possibly originating from the glutathione peroxidase 1 (*GPX1*) gene mapped to 3p13–q12. Mapping of the bovine homolog of *GPX1* may provide further insight into the evolution of HSA 21.

Additional evidence for an ancestral syntenic association of HSA 3 and HSA 21 gene homologs comes from mapping studies in marsupials. In the marsupial *Sminthopsis crassicaudata*, *TF* (HSA 3) is in linkage group U2 with *SOD1* (HSA 21) (31). The significance of this finding cannot be fully addressed at this time, as only a small number of genes have been mapped for this species.

The skeletal physical map of the bovine genome is being established by a variety of classic methods, including the use of hybrid somatic cells and *in situ* hybridization (11, 32). The genetic map of the cow will also be generated by normal procedures, including searching for RFLPs associated with genes of interest and studying the inheritance of these RFLPs in pedigreed families. The determination of the extent of conservation between bovine syntenic groups and human and mouse chromosomes will help direct the generation of a linkage map for bovine chromosomes. The mapping of HSA 21/MMU 16 genes in the cow allows the evaluation of conservation for one important set of genes.

The results discussed here indicate the importance of substantial mapping in mammalian species other than *Homo sapiens* and *Mus musculus*. The question of whether the association of HSA 21 genes with HSA 3 genes seen in the laboratory mouse is recent or ancestral could not be adequately addressed until the mapping of those same genes had been done in at least one other mammalian species such as *Bos taurus*. Mapping of these same genes in additional mammalian species will increase understanding of the rearrangements involved in the evolution of HSA 21, MMU 16, and bovine U10. Comparative gene maping can also allow the prediction of gene order on human chromosomes. From the mapping data presented here, we propose that *RHO* is proximal to *TF* and *CP*, such that one break between these loci would allow the generation of the two bovine chromosomes carrying HSA 3 homologs (Fig. 3).

The identity of the bovine chromosome represented by U10 is still uncertain. Viable trisomies have been reported in cattle, primarily for certain of the smaller chromosomes (33, 34). Because the genetic content of U10 seems as great or greater than MMU 16, there may not be a possibility of viable trisomies for the U10 chromosome in cattle. Nonetheless, identification of the bovine chromosome that is homologous to HSA 21 may be important for the development and evaluation of Down syndrome-like phenotypes in cattle.

We thank the following people for graciously supplying the probes used in this study: *APP* and *ETS2*, R. Reeves (Johns Hopkins University School of Medicine); *COL6A1* and *COL6A2*, M.-L. Chu (Thomas Jefferson University); *CD18*, T. Springer (Dana–Farber Cancer Institute); *GAP43*, R. Neve (Harvard Medical School); *PFKL*, Y. Groner (Weizmann Institute); and *S100B*, R. Dunn (University of Toronto). *TF* was obtained from the American Type Culture Collection. We thank E. Owens and D. W. Threadgill for excellent technical assistance. This work was supported in part by the Texas A&M University Institute of Biosciences and Technology,

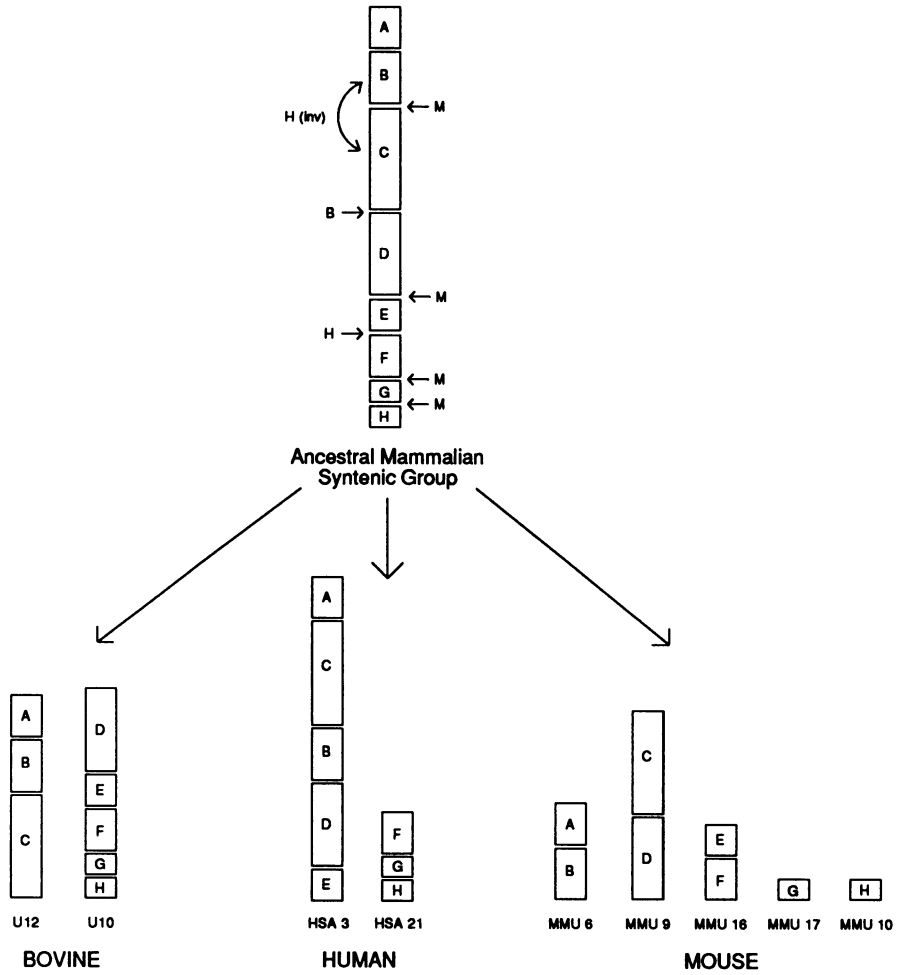

FIG. 4. Proposed scheme for the evolution of HSA 21, MMU 16, and bovine U10 from an ancestral mammalian chromosome. Arrows indicate the location of breakpoints in the ancestral chromosome necessary for the creation of the extant mouse (M), bovine (B), and human (H) chromosomes. Genes represented by each of the boxes are as follows: box A, *RAF1* (D.S.T. and J.E.W., unpublished work); box B, *RHO* (13); box C, *ACY1* (12); box D, *TF* and *CP*; box E, *SST* and *GAP43*; box F, *APP*, *SOD1*, and *ETS2*; box G, *CBS* and *CRYA1*; box H, *S100B*, *CD18*, *COL6A1*, and *COL6A2*. An inversion (inv) has been proposed for the human lineage to account most easily for the association of the B and C block of genes in both the mouse and the bovine (10).

Texas Agricultural Experiment Station Project 6718, and U.S. Department of Agriculture Grants 87-CRSR-2-3126 and 87-CRSR-1-2437. D.S.T. was also supported by a National Needs in Biotechnology Predoctoral Fellowship from the U.S. Department of Agriculture.

1. Carritt, B. & Litt, M. (1989) *Cytogenet. Cell Genet.* **51**, 358–371.
2. Lalley, P. A., Davisson, M. T., Graves, J. A. M., O'Brien, S. J., Roderick, T. H., Creau-Goldberg, N., Hillyard, A. L., Doolittle, D. P. & Rogers, J. A. (1989) *Cytogenet. Cell Genet.* **51**, 503–532.
3. McAvin, J., Patterson, D. & Womack, J. (1987) *Biochem. Genet.* **26**, 9–18.
4. Womack, J. & Cummins, J. (1984) *Cytogenet. Cell Genet.* **37**, 612 (abstr.).
5. Skow, L. C., Womack, J. E., Petresh, J. M. & Miller, W. L. (1988) *DNA* **7**, 143–149.
6. Dietz, A. B. & Womack, J. E. (1989) *J. Hered.* **80**, 410–412.
7. Reeders, S. T. & Hildebrand, C. E. (1989) *Cytogenet. Cell Genet.* **51**, 299–318.
8. Krawetz, S. A., Herfort, M. H., Hamerton, J. L., Pon, R. T. & Dixon, G. H. (1989) *Genomics* **5**, 639–645.
9. Kosik, K. S., Orecchio, L. D., Bruns, G. A. P., Benowitz, L. I., MacDonald, G. P., Cox, D. R. & Neve, R. L. (1988) *Neuron* **1**, 127–132.
10. Naylor, S. L. & Bishop, D. T. (1989) *Cytogenet. Cell Genet.* **51**, 106–120.
11. Nadeau, J. H. (1986) *Genet. Res.* **48**, 175–178.
12. Womack, J. & Moll, Y. (1986) *J. Hered.* **77**, 2–7.
13. Womack, J. E., Threadgill, D. W., Moll, Y. D., Faber, L. K., Foreman, M. L., Dietz, A. B., Tobin, T. C., Skow, L. C., Zneimer, S. M., Gallagher, D. S. & Rogers, D. S. (1989) *Cytogenet. Cell Genet.* **51**, 1109 (abstr.).
14. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
15. Reeves, R. H., Morgan, R., Bendotti, C., Oster-Granite, M. L., Gearhart, J. D. & Coyle, J. T. (1987) *Soc. Neurosci. Abstr.* **13**, 1022.
16. Watson, D. K., McWilliams-Smith, M. J., Kozak, C., Reeves, R., Gearhart, J., Nunn, M. F., Nash, W., Fowle, J. R., III, Duesberg, P., Papas, T. S. & O'Brien, S. J. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 1792–1796.
17. Neve, R. L., Perrone-Bizzozero, N. I., Finklestein, S., Zwiers, H., Bird, E., Kurnit, D. M. & Benowitz, L. I. (1987) *Mol. Brain Res.* **2**, 177–183.
18. Levanon, D., Danciger, E., Dafni, N. & Groner, Y. (1986) *Biochem. Biophys. Res. Commun.* **141**, 374–380.
19. Chu, M.-L., Mann, K., Deutzmann, R., Pribula-Conway, D., Hsu-chen, C.-C., Bernard, M. P. & Timpl, R. (1987) *Eur. J. Biochem.* **168**, 309–317.
20. Dunn, R., Landry, C., O'Hanlon, D., Dunn, J., Allore, R., Brown, I. & Marks, A. (1987) *J. Biol. Chem.* **262**, 3562–3566.
21. Kishimoto, T. K., O'Connor, K., Lee, A., Roberts, T. M. & Springer, T. A. (1987) *Cell* **48**, 681–690.
22. Kraus, J. P., Williamson, C. L., Firgaira, F. A., Yang-Feng, T. L., Munke, M., Francke, U. & Rosenberg, L. E. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 2047–2051.
23. Krawetz, S. A., Connor, W. & Dixon, G. H. (1987) *DNA* **6**, 47–57.
24. Uzan, G., Frain, M., Park, I., Besmond, C., Maessen, G., Trepat, J. S., Zakin, M. M. & Kahn, A. (1984) *Biochem. Biophys. Res. Commun.* **119**, 273–281.
25. Feinberg, A. P. & Vogelstein, B. (1983) *Anal. Biochem.* **132**, 6–13.
26. Tsui, L.-C., Farrall, M. & Donis-Keller, H. (1989) *Cytogenet. Cell Genet.* **51**, 166–201.
27. Threadgill, D. S., Womack, J. E., Kraus, J. P. & Krawetz, S. A. (1989) *Cytogenet. Cell Genet.* **51**, 1090 (abstr.).
28. Reeves, R. H., Gallahan, D., O'Hara, B. F., Callahan, R. & Gearhart, J. D. (1987) *Cytogenet. Cell Genet.* **44**, 76–81.
29. Larsen, B. (1977) *Anim. Blood Groups Biochem. Genet.* **8**, 111–113.
30. McBride, O. W., Mitchell, A., Lee, B. J., Mullenbach, G. & Hatfield, D. (1988) *BioFactors* **1**, 285–292.
31. Graves, J. A. M. (1987) in *Genetic Maps*, ed. O'Brien, S. J. (Cold Spring Harbor Lab., Cold Spring Harbor, NY), Vol. 4, pp. 504–507.
32. Threadgill, D. W., Fries, R., Faber, L. K., Vassart, G., Gunawardana, A., Stranzinger, G. & Womack, J. E. (1990) *Cytogenet. Cell Genet.* **53**, 32–36.
33. Mayr, B., Krutzler, H., Auer, H., Schleger, W., Sasshofer, K. & Glawischnig, E. (1985) *Cytogenet. Cell Genet.* **39**, 77–79.
34. Mayr, B., Schellander, K., Auer, H., Tesarik, E., Schleger, W., Sasshofer, K. & Glawischnig, E. (1987) *Cytogenet. Cell Genet.* **44**, 229–230.